sured by subtracting the luminescence value of the medium alone. When the RLU value (ATP measurement, luminescence intensity) of the culture medium was 100%, the relative value of the filtrate was taken as the filtrate outflow cells and the relative value of the collection solution was taken as the collection rate. The measurement results are shown in Table 13.

(Measurement of Number and Average Diameter of Cell Aggregates)

[0356] The cell aggregates were well dispersed. A part of the culture medium used in the test was placed in a 12 well plate, and the size of the cell aggregates with a diameter of 50 μm or more in the culture medium was measured. In the measurement of the size of the cell aggregates, the equivalent-circle-diameter of the cell aggregate was measured, and the mean thereof was taken as the average diameter. The measurement results are shown in Table 13.

(Evaluation of Undifferentiated State)

[0357] A part of the cells on day 7 of culture was separated and dissociated into single cells by TripLE Select (manufactured by ThermoFisher Scientific, #12563011) treatment, suspended in 4% PFA solution, and immobilized by allowing to stand for 15 min.

[0358] The immobilized cells were stained by suspending in anti-TRA-1-60 antibody (BD, #560193) and anti-SSEA-4 antibody (BD, #560796) solution and allowing to stand for 30 min. The cells were washed with PBS solution containing 2% FBS, and the expression of TRA-1-60 and SSEA-4, which are indicators of the undifferentiated state of iPS cells, was measured using Fortessa X-20(BD). The measurement results are shown in Table 14.

TABLE 13

proliferation rate (fold)	9.4
collection rate (%)	72.9
cells that passed mesh (%)	8.8
cell aggregate average diameter (μm)	162.1

TABLE 14

	proportion of TRA-60 positive, SSEA-4 positive cell population (%)
after 3 passages	77.3

[0359] As shown in Tables 13 and 14, it was clarified that undifferentiated culture can be performed while maintaining a good proliferation rate in the cell culture system (that is, cell culture system using a closed system flow path).

INDUSTRIAL APPLICABILITY

[0360] According to the present invention, it is possible to perform division of cell aggregates and further suspension culture of the divided cells in a closed system, and further repeat division and culture of cell aggregates while maintaining the closed system, and preferably perform proliferation of cell aggregates. In addition, it is possible to collect the proliferated cell aggregates while maintaining the closed system.

[0361] This application is based on a patent application No. 2018-148042 filed in Japan (filing date: Aug. 6, 2018) and a patent application No. 2018-170103 filed in Japan (filing date: Sep. 11, 2018), the contents of which are incorporated in full herein.

EXPLANATION OF SYMBOLS

[0362] **10** first container
[0363] **20** divider
[0364] **22** mesh structure
[0365] S**1** supply source
[0366] P**1** conduit
[0367] P**2** conduit
[0368] P**3** conduit
[0369] F**1** liquid feeding device

1. A cell culture system for dividing and subculturing a cell aggregate, comprising at least:

a divider for dividing a cell aggregate to be divided which is supplied together with a liquid medium from a supply source into smaller cell aggregates; and

a first container for suspension culturing the cell aggregates divided by the divider;

wherein,

the divider has an inlet, an internal dividing conduit, and an outlet, the inlet is constituted such that the cell aggregate to be divided and the liquid medium are received from the supply source into the dividing conduit, the dividing conduit is provided with a mesh structure to divide the cell aggregate to be divided, the cell aggregate to be divided is divided when passing through the mesh structure together with the liquid medium, and the outlet is connected to the first container so as to deliver the divided cell aggregates to the first container, and

the first container has a constitution for receiving the divided cell aggregates and the liquid medium and sending out the cell aggregates suspension cultured in the first container.

2. The cell culture system according to claim **1**, wherein the first container is a flexible cell culture bag.

3. The cell culture system according to claim **1**, wherein the mesh structure is a mesh woven with wire.

4. The cell culture system according to claim **1**, wherein

the mesh structure is a porous film with many through-holes disposed on the film surface, the mesh structure comprises many through-holes penetrating the predetermined region in the film thickness direction, and a beam part serving as a partition between the through-holes,

the through-holes have an opening shape of a size permitting passage of the smaller cell aggregates,

and the beam part is a remainder after subtracting the through-hole from the main body part in the predetermined region, is a part that cuts the cell aggregates to be divided, and is integrally connected to form a network.

5. The cell culture system according to claim **4**, wherein a cross-sectional shape in the perpendicular longitudinal direction of the beam part is a rectangle, or two corners on the inlet side of the rectangle have a round shape.

6. The cell culture system according to claim **4**, wherein said many through-holes have opening shapes of quadrangles congruent with each other, and said beam parts are connected to each other in an orthogonal lattice pattern, or